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## Physiological Effects of Low-Magnesium Feeding in the Common Carp, *Cyprinus carpio*

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**ABSTRACT** Common carp (*Cyprinus carpio*) initially weighing about 100 g were fed a low-magnesium diet for 17 weeks. The fish survived this treatment and had a healthy appearance. However, the fish became hypomagnesemic and their growth rate decreased. The bone sodium concentration increased and the magnesium concentration decreased. It thus appears that in carp, under dietary magnesium deficiency, magnesium is mobilized from internal stores and that magnesium in the bone mineral is replaced by sodium. Low-magnesium fed carp further showed a decreased branchial  $\text{Na}^+/\text{K}^+$ -ATPase specific as well as total activity. Unexpectedly, the opercular chloride cell density increased. We tentatively conclude that more and new chloride cells with a lower  $\text{Na}^+/\text{K}^+$ -ATPase content develop during this treatment. We further conclude that adult carp require dietary magnesium for the maintenance of their magnesium balance as well as for the proper functioning of branchial ionoregulatory mechanisms. Low-magnesium fed carp do not acclimate but appear to tolerate a dietary magnesium deficiency. © 1992 Wiley-Liss, Inc.

Magnesium is an essential element and it plays a pivotal role in the physiology of vertebrates. Freshwater fish can take in magnesium via branchial and intestinal routes. In tilapia (*Oreochromis mossambicus*) intestine, a net mucosa-to-serosa magnesium flux was demonstrated (van der Velden et al., '90), and these experiments have given direct evidence for a role of the intestine in magnesium absorption in fish. In experiments with adult freshwater tilapia (about 200 g) fed low-magnesium diets (Mg content:  $1 \text{ mmol.kg}^{-1}$  versus  $30 \text{ mmol.kg}^{-1}$  in the control diet) we have shown that this species can survive low-magnesium feeding and keeps on growing without depleting the magnesium pools of scales, bone, or muscle (van der Velden et al., '91b). It was concluded that the adult tilapia has acclimational mechanisms to cope with low-magnesium feeding and that the important ionoregulatory hormone prolactin may play a pivotal role in this acclimation process (van der Velden et al., '92).

Carp take up at least 84% of their magnesium from the food via the intestinal tract and maximally 16% directly from the water via the integument (gills, skin); drinking (if these fish drink at all under normal circumstances) will contribute 10% at most to this integumental uptake of magnesium and is, in this respect, assumed to be insignificant for the physiology of the fish (van der Velden, '91a). Dietary

magnesium requirements of teleosts have been studied by a number of workers. For small carp (initial weight: 2.8 g) a magnesium requirement of around  $20 \text{ mmol.kg}^{-1}$  food has been reported for optimum growth (Ogino and Chiou, '76). Diet studies of this kind on adult carp are to our knowledge lacking. We here address the question whether the adult carp is capable to cope with low-magnesium feeding conditions. We have determined growth rate, the elemental composition of plasma and bone, and analyzed some ion regulatory mechanisms. The study of ion regulatory mechanisms included the evaluation of prolactin cell activity, opercular chloride cell density, and branchial  $\text{Na}^+/\text{K}^+$ -ATPase content. Branchial  $\text{Na}^+/\text{K}^+$ -ATPase activity is concentrated in the chloride cells (Hootman and Philpott, '79; Mayer-Gostan et al., '87). The hematocrit was determined as a parameter for drastic changes in ion regulation (Milligan and Wood, '82) and the health condition of the fish.

### MATERIALS AND METHODS

Adult carp (*Cyprinus carpio*) were kept in all-glass aquaria filled with 450 liter Nijmegen tap water.

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The magnesium concentration of the water ranged from 0.17 to 0.23 mM, the pH was around 7.6, and the temperature was 25°C. The water in the aquarium was filtered by recirculation through an 800 liter biological filter system and continually refreshed with 300 liter water per day. The photoperiod was automatically controlled (12 h light: 12 h dark). The fish were weighed weekly. We used a PM34 Delta Range balance (Mettler, Tiel, The Netherlands) that allows weighing of free swimming fish and minimizes handling stress. The balance precision was set to 0.1 g. Fish weights were rounded off to the nearest gram.

The fish were fed daily 2% of the total wet weight of the group by means of an automated food dispenser. A batch of 72 fish was randomly divided into two groups of 36 fish each, weighing  $90 \pm 14$  g (mean  $\pm$  s.d., experimental group) and  $88 \pm 14$  g (mean  $\pm$  s.d., control group). Both groups were fed the control diet for two weeks. Then the experiment was started by providing the experimental group (weighing now  $109 \pm 22$  g) a low-magnesium diet; the control group (weighing  $103 \pm 16$  g) received control diet. The duration of the experiment was 17 weeks. The composition of the control diet has been described previously (van der Velden et al., '91c). In the experimental diet, MgO and  $\text{MgSO}_4 \cdot 2\text{H}_2\text{O}$  were completely replaced by glucose monohydrate. The total magnesium concentrations in the control and the low-magnesium diets were  $30 \pm 5$  and  $1 \pm 1$  mmol.kg<sup>-1</sup> diet, respectively. Both diets were produced by Hope Farms (Woerden, The Netherlands).

With 2 to 5 week intervals (depending on the type of parameter that was determined) fish were sampled at random from the populations for further analyses. The randomness of sampling was ascertained by testing the body weight distribution in the populations for normal distribution (see below).

Mixed arterial and venous blood was collected by puncture of the caudal vessels using an ammonium heparin-rinsed tuberculin syringe fitted with a 21G needle. Blood was separated into plasma and cells by centrifugation at 9000g for 3 min. Part of the blood plasma was ultrafiltered using filter-units (ultrafree-MC UFC3 TGCOO, Millipore, Bedford, MA, USA) with a 10 kDa molecular cut-off. The magnesium and calcium concentrations of blood plasma and ultrafiltered blood plasma were determined with commercial kits (numbers 595 and 587 for calcium and magnesium, respectively; Sigma Chemical Company, St. Louis, MO). The magnesium assay is based on the colorimetric assay of a complex of magnesium and calmagite (1-[1-hydroxy-4-methyl-

2-phenylazo]-2-naphthol-4-sulphonic acid) at 520 nm. The calcium assay is based on the colorimetric assay of a complex of calcium and cresolphthaleine complexone at 595 nm. The osmolality of blood plasma was determined with a micro-osmometer (Roebling Inc.) using distilled water and a 300 mOsmol.kg<sup>-1</sup> standard (Sigma Chemical Company) as reference. Hematocrit was assessed after 10 min centrifugation of heparinized blood samples in 75  $\mu$ l glass capillaries.

The fish were killed by transection of the spinal cord. Then the fish was microwave-cooked for three minutes and three vertebrae of the caudal spinal cord were collected and freed of adherent muscle. The bone tissue was weighed, lyophilized, and weighed again to assess the water content of the sample. The magnesium, calcium, and sodium concentrations of the skeletal bone were determined by instrumental neutron activation analysis (van der Velden et al., '89). In short, lyophilized samples of 5 to 150 mg were encapsulated in 0.5 ml polyethylene vials and irradiated with a thermal neutron flux of  $1.3 \cdot 10^{17}$  m<sup>-2</sup>.s<sup>-1</sup> for 20 sec. After a 20 sec delay, the samples were measured with a gamma-ray spectrometer consisting of a lead-shielded High Purity Germanium detector (Ortec, Oak Ridge, TN, USA), a spectroscopy amplifier (model 673, Ortec), and a multichannel analyser (TN 1710, Tracor Northern, Middleton, WI, USA) including a 200 MHz analog to digital converter. Magnesium was measured via <sup>27</sup>Mg (half-life 9.46 min,  $E_\gamma$  = 844 and 1014 keV), calcium via <sup>49</sup>Ca (half-life 8.72 min,  $E_\gamma$  = 3084 keV), and sodium via <sup>24</sup>Na (half-life 15.03 h,  $E_\gamma$  = 1368 and 2754 keV). Calibration was performed via zinc flux monitors irradiated simultaneously with the samples. After every eight samples, a sample of "Standard Reference Material 1571 Orchard Leaves" and of "Bowens Kale" were included. The determined values matched these reference materials and indicate that no systematic errors in the assay occurred.

The complexity of the gill structure makes it difficult to estimate the number of chloride cells therein. We therefore present the number of chloride cells per surface area in the opercular membrane, which is considered to reflect the branchial chloride cell content (Foskett et al., '81). To estimate the chloride cell density in the inner opercular membrane, a complete operculum was excised and transferred to well-aerated water containing  $2 \mu\text{mol.l}^{-1}$  2-(dimethylaminostyryl)-1-ethylpyridinium-dine (DASPEI; ICN Biomedicals, Santa Ana, CA, USA), which stains the mitochondria-rich chloride cells (Bereither-Hahn, '76). After 45 min the

operculum was rinsed three times for 5 min in water and examined with a Zeiss epifluorescence microscope at a magnification of  $\times 250$ . Cells were counted in 20 different squares of the opercular epithelium with a total surface area of  $5 \text{ mm}^2$  per fish.

The branchial epithelium was scraped off the gill arches onto an ice-cooled glass plate with a microscope slide. The collected soft tissue was transferred to an isotonic buffer containing 300 mM sucrose, 20 mM Hepes/T-ris, pH 7.4, 0.1 mM EDTA, 0.1 mM dithiothreitol (Sigma Chemical Company),  $100 \text{ U.ml}^{-1}$  aprotonin (Sigma Chemical Company) and  $50 \text{ U.ml}^{-1}$  Na-heparin. The scrapings were homogenized in this buffer using a tight glass-to-glass Potter homogenizer. During the homogenization step disrupted membranes tend to reseal and due to this process substrate sites may become masked resulting in an apparent decrease of enzymic activity. Substrate accessibility was optimized (Flik et al., '90b) by treatment with the detergent saponin ( $20 \mu\text{g.ml}^{-1}$  membrane protein). The  $\text{Na}^+/\text{K}^+$ -ATPase activity was determined by the method of Bonting and Caravaggio ('63). In short, the  $\text{Na}^+/\text{K}^+$ -ATPase activity was defined as the  $\text{K}^+$ -dependent and ouabain-sensitive ATPase activity, determined as the difference in membrane induced ATP-hydrolysis in a medium containing both  $\text{Na}^+$  and  $\text{K}^+$  and in a medium containing no  $\text{K}^+$  but  $1 \text{ mg.ml}^{-1}$  ouabain (G-strophantin, Boehringer, Mannheim, Germany). Protein was determined with a 'Bradford-type' protein assay (Biorad, Richmond, CA, USA) using bovine serum albumin as a reference protein. Enzymic activities are presented as specific activity expressed as the amount of inorganic phosphate liberated from ATP per h per mg membrane protein ( $\mu\text{mol.h}^{-1}.\text{mg prot.}^{-1}$ ). The total ATPase activity was calculated as the product of the specific activity and the total protein content of the branchial epithelial homogenate ( $\mu\text{mol.h}^{-1}$ ).

A measure for the prolactin cell activity is the rate of uptake and incorporation of tritiated amino acids by freshly dissected pituitary glands in vitro, as described previously (Wendelaar Bonga et al., '88; Flik et al., '89). Briefly, the freshly dissected rostral pars distalis ("prolactin lobe", RPD) of the pituitary gland was preincubated for 30 min in Hank's Balanced Salt Solution (Sigma Chemical Company) and subsequently incubated for 4 h in  $50 \mu\text{l}$  of the solution containing  $1 \text{ MBq}$  [ $^3\text{H}$ ]-lysine or [ $^3\text{H}$ ]-leucine (Amersham International plc, Amersham, U.K.). Subsequently the RPD was homogenized and the homogenate and the products released to the incubation media (collected by trichloroacetic acid precipitation) were subjected to sodium

dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE; for detailed description of the procedures see Wendelaar Bonga et al., '83). After separation the prolactin band was identified by Western blotting, using an antiserum raised in rabbits against salmon (*Oncorhynchus nerka*; a generous gift from Dr. B.A. McKeown, Vancouver, B.C.). At the light microscopic immunohistochemical level this antiserum highly specifically stains the rostral pars distalis cells that are considered the prolactin cells (data not shown). The newly synthesized prolactin was identified by an immunoprecipitation technique (Flik et al., '90a) with the same antiserum and Protein-A Sepharose CL-4B (Pharmacia) as a solid-phase immunoadsorbent. The total amount of labeled (newly synthesized) prolactin present in the RPD and medium was determined by densitometry of the prolactin bands in fluorographs of the gels. The molecular weight of the proteins was estimated by comparison with prestained 'low molecular weight markers' (Biorad) or  $^{14}\text{C}$ -methylated protein molecular weight markers (Amersham International plc).

#### Data handling

Data are presented as mean values  $\pm$  standard deviation of the mean. Data were statistically analysed by Student's *t*-test. The body weights of the experimental and control group were statistically tested for a normal distribution at the start and at the end of the experiment, using the Kolmogorov-Smirnov test. Statistical significance was accepted at  $P \leq 0.05$  unless indicated otherwise.

#### RESULTS

During the experiments no mortality was observed and all fish appeared healthy. Both groups were steadily growing from  $103 \pm 16 \text{ g}$  ( $n = 36$ ) to  $415 \pm 28 \text{ g}$  ( $n = 11$ ) and from  $109 \pm 22 \text{ g}$  ( $n = 36$ ) to  $356 \pm 60 \text{ g}$  ( $n = 11$ ) after 17 weeks for the control and experimental group, respectively. The body weights of the experimental and control group at the beginning and end of the experiment were normally distributed (data not shown) and therefore, the use of the Student's *t*-test for the assessment of the significance of differences in body weights is justified. The body weight ranges at the start of the experiment were comparable; the range in body weights at the end of the experiment was significantly ( $P < 0.01$ ) larger in the experimental group ( $262\text{--}444 \text{ g}$ ) than in the control group ( $355\text{--}450 \text{ g}$ ). A mean decrease in the growth rate over this 17 week time period of 21% ( $P < 0.01$ ) can be calculated for carp fed the low-magnesium diet.

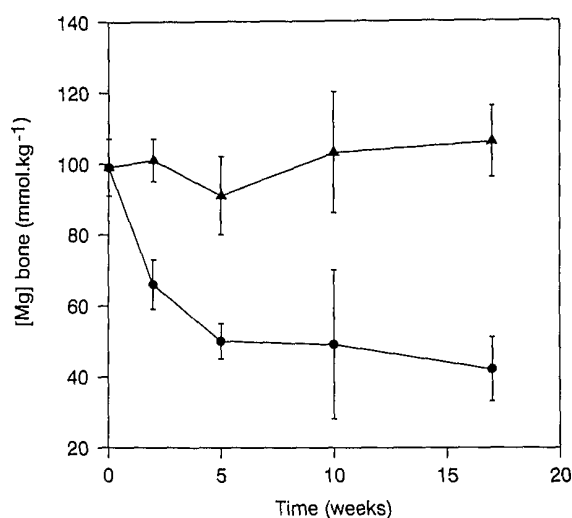


Fig. 1. Bone magnesium content in carp fed a low-magnesium ( $1 \text{ mmol.kg}^{-1}$ ) diet (●—●) or a control ( $30 \text{ mmol.kg}^{-1}$ ) diet (▲—▲). Significant differences occurred as early as two weeks after the start of the experiment. No recovery in bone magnesium content was observed in the experimental fish.

The magnesium concentration in vertebral bone of the control group did not change significantly during the experimental time period; the mean value was  $99 \pm 19 \text{ mmol.kg}^{-1}$  ( $n = 25$ ). However, in the experimental group, this concentration had decreased ( $P < 0.025$ ) after two weeks to  $66 \pm 7 \text{ mmol.kg}^{-1}$  ( $n = 5$ ) and to  $42 \pm 9 \text{ mmol.kg}^{-1}$  ( $P < 0.01$ ;  $n = 5$ ) after 17 weeks (Fig. 1). The calcium concentrations in the bone of both the control and experimental group did not change in time ( $P > 0.15$ ) and the mean values in time were  $3,800 \pm 250 \text{ mmol.kg}^{-1}$  ( $n = 25$ ) and  $3,850 \pm 350 \text{ mmol.kg}^{-1}$  ( $n = 25$ ), respectively. The sodium concentration of the bone after 2 weeks was comparable for control and experimen-

tal carp, viz.  $188 \pm 6 \text{ mmol.kg}^{-1}$  ( $n = 5$ ) and  $198 \pm 21 \text{ mmol.kg}^{-1}$  ( $n = 5$ ), respectively. However, after 17 weeks the sodium concentration had increased ( $P < 0.02$ ) in the low magnesium fed carp, viz. from  $217 \pm 8 \text{ mmol.kg}^{-1}$  ( $n = 5$ ) in the controls to  $234 \pm 9 \text{ mmol.kg}^{-1}$  ( $n = 5$ ) in the experimentals. The mean water content of the vertebrae of control and experimental carp was not significantly different, viz. ( $52 \pm 4\%$ ) ( $n = 25$ ) and ( $51 \pm 3\%$ ) ( $n = 25$ ), respectively.

The mean concentrations of magnesium and calcium in the plasma and its ultrafiltrate, the osmolarity of the plasma, and the blood hematocrit of control and low-magnesium fed carp at week 10 and week 17 are given in Table 1. Values for hematocrit and plasma osmolarity did not change. Total plasma and ultrafiltrable plasma magnesium concentrations were significantly reduced. Plasma calcium was slightly but significantly ( $P < 0.05$ ) reduced at week 17, and plasma ultrafiltrable calcium was reduced both at week 10 and 17.

Table 2 shows the branchial  $\text{Na}^+/\text{K}^+$ -ATPase specific and total activities and the opercular chloride cell density in control and experimental fish at week 10 and 17. After 10 and 17 weeks the branchial  $\text{Na}^+/\text{K}^+$ -ATPase specific activities in the experimental fish amounted to 35 and 45%, respectively, of the values in the controls; the corresponding total activities amounted to 35 and 40%, respectively. The number of opercular chloride cells in the experimental fish had more than doubled after 17 weeks.

Based on the ultrastructure of the prolactin cells of both groups no indications for changes in activity were found (Fig. 2). An antiserum raised against Pacific salmon recognizes a  $20 \pm 0.8$  ( $n = 5$ ) kilodalton protein species in extracts of carp pituitary

TABLE 1. Plasma ion composition in low-magnesium fed carp<sup>1</sup>

	Week 10		Week 17	
	Control	Experimental	Control	Experimental
Plasma				
-Mg	$0.78 \pm 0.10$	$0.33 \pm 0.19^{***}$	$0.95 \pm 0.03$	$0.35 \pm 0.08^{***}$
-Mg <sup>2+</sup>	$0.66 \pm 0.08$	$0.30 \pm 0.14^{***}$	$0.73 \pm 0.04$	$0.19 \pm 0.05^{***}$
-Ca	$2.44 \pm 0.09$	$2.30 \pm 0.15$	$2.44 \pm 0.06$	$2.32 \pm 0.08^*$
-Ca <sup>2+</sup>	$1.80 \pm 0.10$	$1.62 \pm 0.07^{**}$	$1.82 \pm 0.05$	$1.71 \pm 0.07^*$
-Osmolarity	$260 \pm 4$	$263 \pm 4$	$258 \pm 3$	$257 \pm 1$
-Hematocrit	$23 \pm 1$	$25 \pm 1$	$23 \pm 2$	$22 \pm 2$

<sup>1</sup>Plasma ion concentrations (in mM), plasma osmolarity (in mOsm.kg<sup>-1</sup>), and hematocrit value (in % of the total blood volume) in carp after 10 and 17 weeks feeding on a low-magnesium diet ( $1 \text{ mmol.kg}^{-1}$ ) and a control diet ( $30 \text{ mmol.kg}^{-1}$ ). Mean values  $\pm$  standard deviation are given, the number of observations is 5.

\*  $P < 0.05$ .

\*\*  $P < 0.02$ .

\*\*\*  $P < 0.01$ .

TABLE 2. Branchial  $\text{Na}^+/\text{K}^+$ -ATPase activity and opercular chloride cell densities in low-magnesium fed carp<sup>1</sup>

	Week 10		Week 17	
	Control	Experimental	Control	Experimental
$\text{Na}^+/\text{K}^+$ -ATPase				
-specific activity	23.3 $\pm$ 4.1	8.2 $\pm$ 4.3***	29.5 $\pm$ 8.7	13.2 $\pm$ 6.4***
-total activity	191 $\pm$ 48	67 $\pm$ 24***	355 $\pm$ 85	141 $\pm$ 66***
Chloride cell density	25 $\pm$ 6	38 $\pm$ 14	27 $\pm$ 5	59 $\pm$ 7***

<sup>1</sup> $\text{Na}^+/\text{K}^+$ -ATPase specific activity (in  $\mu\text{mol P}_i \cdot \text{h}^{-1} \cdot \text{mg}^{-1}$  protein), total activity (in  $\mu\text{mol P}_i \cdot \text{h}^{-1}$ ), and opercular chloride cell density (number  $\cdot \text{mm}^{-2}$ ) in carp feeding for 10 and 17 weeks on a diet containing 1  $\text{mmol} \cdot \text{kg}^{-1}$  Mg (experimentals) and 30  $\text{mmol} \cdot \text{kg}^{-1}$  Mg (controls).

\*\*\*  $P < 0.01$ .

glands and a  $20 \pm 0.9$  ( $n=10$ ) kilodalton labeled protein species after labeling of the glands with radiolabeled amino acids (Fig. 3). Prolactin cell activity as indicated by the rate of synthesis of newly formed prolactin did not notably change. After 10 weeks the values (in arbitrary units %) for the 20 kDa peak areas were  $100 \pm 32$  and  $66 \pm 30$  ( $n=5$ ,  $P > 0.075$ ) for controls and experimentals, respectively;

after 17 weeks the values were  $100 \pm 38$  and  $107 \pm 31$  ( $n=5$ ,  $P > 0.15$ ), respectively.

### DISCUSSION

Carp feeding for 17 weeks on a low-magnesium diet become hypomagnesemic and mobilize magnesium from their bone. Furthermore, the decreased branchial  $\text{Na}^+/\text{K}^+$ -ATPase activity and the in-

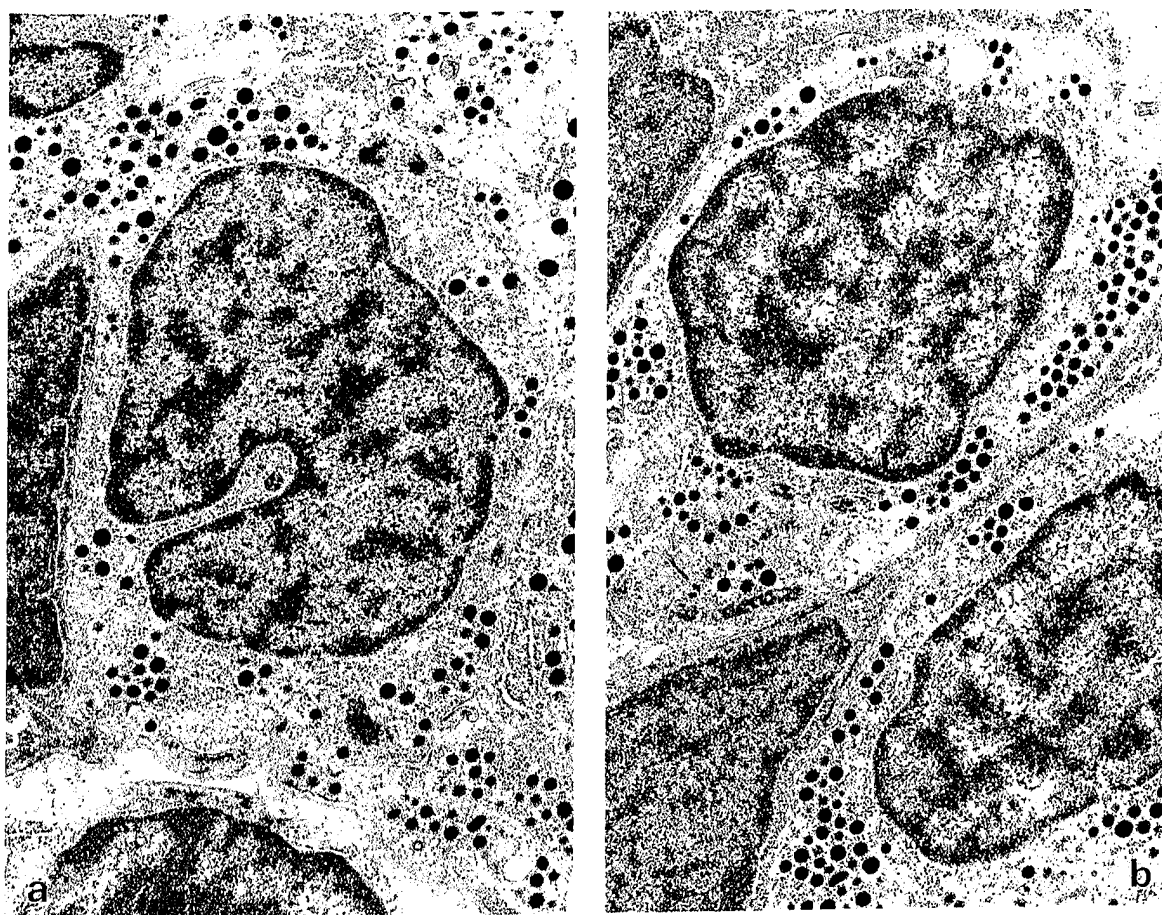


Fig. 2. Electron micrographs of prolactin cells of carp;  $\times 10,800$ . The cells show nuclei surrounded by small rings of cytoplasm containing many electron dense granules. The struc-

ture is similar in controls (a) and in carp put on a low-magnesium diet for 17 weeks (b).

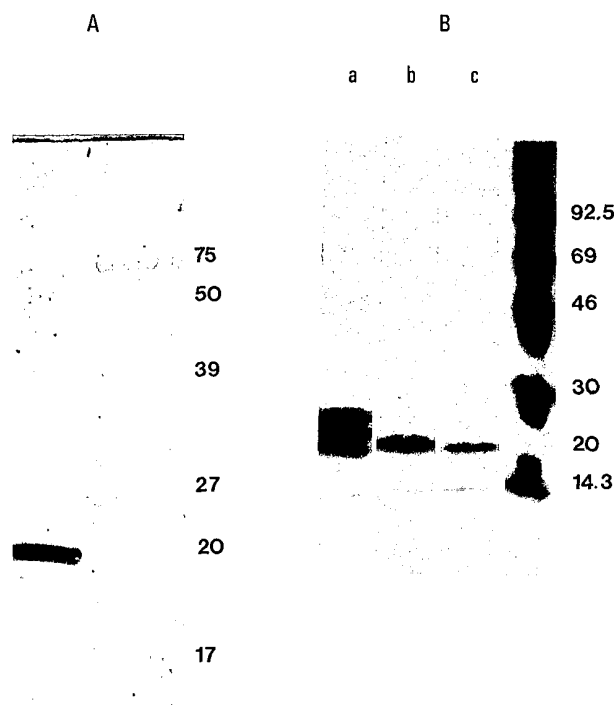


Fig. 3. SDS-PAGE analysis of carp pituitary extracts. A: Western blot of pituitary extract (4.28  $\mu$ g protein). The sample was separated by 1-dimensional gel electrophoresis under reducing conditions. Transfer to nitrocellulose was complete after 1 h at 100 V. Nitrocellulose was probed with a 1:5,000 diluted antiserum against Pacific salmon prolactin antiserum, followed by peroxidase-conjugated goat anti-rabbit IgG. Prestained markers were used for calibration. B: Fluorograph of [ $^3$ H]-lysine labeled products newly synthesized by carp pituitary glands in vitro during a 4 h incubation period; a, tissue extract; b, products released to the medium; c, immunoprecipitated newly synthesized products from carp rostral pars distalis labeled with [ $^3$ H]-leucine during a 4 h incubation in vitro. [ $^{14}$ C]-labeled protein markers were used for calibration.

creased opercular chloride cell density indicate that a low magnesium diet disturbs the ionoregulation at the level of the gills. We conclude from the absence of mortality and of notable changes in hematocrit that the health of the fish was not seriously affected by the low-magnesium diet. This conclusion is further supported by the observation that the fish continued to grow. Thus for survival carp are not fully dependent on a strict control of the plasma magnesium levels. They tolerated the lowered magnesium levels in the blood and in other tissues for at least four months.

The values for ion content of plasma and bone tissue determined for the control carp are in good agreement with earlier reports on this species. We found a mean magnesium concentration in blood plasma and in plasma ultrafiltrate of  $0.94 \pm 0.18$

mM ( $n=15$ ) and  $0.73 \pm 0.11$  mM ( $n=15$ ), respectively. Previously we reported a magnesium concentration in blood plasma and in ultrafiltered plasma of  $0.96 \pm 0.06$  mM and  $0.61 \pm 0.01$  mM, respectively (van der Velden et al., '91a). Hunn ('72), Houston ('85), and Jensen ('90) found magnesium concentrations in carp blood plasma of  $0.93 \pm 0.05$  mM,  $1.15 \pm 0.16$  mM, and  $0.94 \pm 0.12$  mM, respectively. We found a blood plasma calcium concentration of  $2.20 \pm 0.44$  mM ( $n=15$ ). Earlier reports give values of  $1.99 \pm 0.05$  (Hunn, '72) and between 2.6–3.3 mM (Nanba et al., '87). The plasma osmolarity of  $260 \pm 4$  mOsm.kg $^{-1}$  is close to that reported by Nanba et al. ('87), viz. around 250 mOsm.l $^{-1}$ . We found a magnesium and calcium concentration in the vertebrae of  $99 \pm 19$  mmol.kg $^{-1}$  ( $n=25$ ) and  $3,820 \pm 270$  mmol.kg $^{-1}$  ( $n=25$ ), respectively. Ogino and Chiou ('76) found in carp, fed a (control) diet with a magnesium concentration comparable to that of the experiments described here, values of about 105 and 3,120 mmol.kg $^{-1}$  for magnesium and calcium, respectively. From this comparison of the elemental composition of important magnesium and calcium pools in the fish, we conclude that our control diet does not disturb the magnesium or calcium balance of carp and may be considered as a sufficient diet.

The decrease in the magnesium concentration of the vertebrae found in carp on a low-magnesium diet is in agreement with a report by Ogino and Chiou ('76), who found for small carp (around 8 g) fed a diet containing 3.3 mmol magnesium per kg a decrease in bone from 105 to 64 mmol.kg $^{-1}$  after 4 weeks. Apparently, carp can mobilize magnesium from their bone compartment. Moreover, these data suggest that the regulation of storage of these ions occurs by independent mechanisms. A decrease in the magnesium concentration of bone was also observed in some other freshwater teleosts, such as rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*) and guppy (*Poecilia reticulata*), fed a low-magnesium diet (Gatlin et al., '82; Knox et al., '83; Ogino et al., '78; Shim and Ng, '88). An increase of the sodium concentration in bone was observed in low-magnesium fed rainbow trout and guppy (Knox et al., '83; Shim and Ng, '88). Dietary depletion of magnesium in higher vertebrates such as rats and dogs also resulted in a decrease of the magnesium concentration in plasma and bone and in an increase of the sodium concentration in bone (George and Heaton, '75; Geven et al., '88).

The control fish take in with their food per day an average of 155  $\mu$ mol magnesium (2% food per day



on weight basis, food Mg content  $30 \text{ mmol.kg}^{-1}$ , average weight 259 g). Assuming an overall magnesium concentration in the carp of  $13 \text{ } \mu\text{mol.g}^{-1}$  (van der Velden et al., '91a), we calculate that these fish require  $34 \text{ } \mu\text{mol.day}^{-1}$  for growth. Apparently the food magnesium is plentiful. However, under low-magnesium feeding conditions the mean quantity of magnesium offered via the food is only  $5 \text{ } \mu\text{mol}$  per day for a fish of comparable weight. Obviously this low-magnesium diet is not enough to supply the magnesium required for growth at a rate found in the control fish and this may explain the decrease in growth rate observed in the experimental fish. Likely carp fed the low-magnesium diet depend on the intake of magnesium from the water, a source which is of minor importance when magnesium sufficient food is offered (van der Velden et al., '91a). The frank mobilization of magnesium from the bone (discussed above) suggests that the carp can not absorb (the essentially limitless) magnesium from the water.

A paradoxical observation is the decreased branchial  $\text{Na}^+/\text{K}^+$ -ATPase activity and the increased opercular chloride cell density. For the tilapia, *Oreochromis mossambicus*, it was shown that upon exposure to acidified water the increased numbers of opercular chloride cells correlate positively with the numbers of these cells in the gills (Wendelaar Bonga et al., '90). Also the  $\text{Na}^+/\text{K}^+$ -ATPase content had increased in both the opercular and branchial epithelium upon exposure of these fish to acidified water. Assuming that in the carp a similar correlation exists between the opercular and branchial chloride cells, we predict from the increased numbers of opercular chloride cells in the low-magnesium fed carp an increased number of branchial chloride cells as well. In these fish then increased numbers of chloride cells would go together with decreased  $\text{Na}^+/\text{K}^+$ -ATPase activity, a situation deviating from that observed in tilapia. The hypothesis to test in the future is that new chloride cells develop in the gills of these fish with a markedly different enzymic make-up. In general however, the data point to a dietary influence on branchial ionoregulatory mechanisms. A similar conclusion was drawn for tilapia fed the same diet (van der Velden et al., '92). However, the responses of carp to a low-magnesium diet differ in several aspects from those of the tilapia (van der Velden et al., '92, '91b): low-magnesium fed tilapia also grow slower than their controls but in contrast to the carp no changes were detected in the magnesium concentration of the bone or the blood plasma. Furthermore, in tilapia a significant increase of the prolactin

cell activity was observed, whereas in carp prolactin cell activity had not changed. Tilapia showed responses that characterize these fish as an effective acclimator to changes in environmental conditions (van der Velden et al., '92). Conversely, as judged from the prolonged hypomagnesemia, carp react more as a tolerator to low-magnesium feeding. Tolerance has been implied earlier for carp as a strategy to cope with other strenuous conditions such as anoxia (van den Thillart et al., '89).

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## LITERATURE CITED

- Bereither-Hahn, J. (1976) Dimethylaminostyrylmethylpyridiniumiodine as a fluorescent probe for mitochondria in situ. *Biochim. Biophys. Acta*, 423: 1-14.
- Bonting, S.L., and L.L. Caravaggio (1963) Studies on  $\text{Na}^+/\text{K}^+$ -activated ATPase. V. Correlation of enzyme activity with cation fluxes in tissues. *Arch. Biochem. Biophys.*, 101: 37-71.
- Flik, G., T. Labedz, J.A.M. Neelissen, R.G.J.M. Hanssen, S.E. Wendelaar Bonga, and P.K.T. Pang (1990a) Rainbow trout corpuscles of Stannius: Stanniocalcin synthesis in vitro. *Am. J. Physiol.*, 258: R1157-R1164.
- Flik, G., Th.J.M. Schoenmakers, J.A. Groot, C.H. van Os, and S.E. Wendelaar Bonga (1990b) Calcium absorption by fish intestine: The involvement of ATP- and sodium-dependent calcium extrusion mechanisms. *J. Membr. Biol.*, 113: 13-22.
- Flik, G., J.A. Vandervelden, H.C.M. Seegers, and Z. Kolar, and S.E. Wendelaar Bonga (1989) Prolactin cell activity and sodium fluxes in tilapia (*Oreochromis mossambicus*) after long-term acclimation to acid water. *Gen. Comp. Endocrinol.*, 75: 39-45.
- Foskett, J.K., C.D. Logsdon, T. Turner, T.E. Machen, and H.A. Bern (1981) Differentiation of the chloride extrusion mechanisms during seawater adaptation of a teleost fish, the cichlid *Sarotherodon mossambicus*. *J. Exp. Biol.*, 93: 209-224.
- Gatlin, D.M., E.H. Robinson, W.E. Poe, and R.P. Wilson (1982) Magnesium requirement of fingerling channel catfish and signs of magnesium deficiency. *J. Nutr.*, 112: 1182-1187.
- George, G.A., and F.W. Heaton (1975) Changes in cellular composition during magnesium deficiency. *Biochem. J.*, 152: 609-615.
- Geven, W.B., G.M. Vogels-Mentink, J.L. Willems, T. de Boo, W. Lemmens, and L.A.H. Monnens (1988) Experimental magnesium depletion in the dog. Influence on the magnesium content of mononuclear cells, erythrocytes, muscle and bone. *Mag. Bull.*, 10: 45-50.
- Hootman, S.R., and C.W. Philpott (1979) Ultracytochemical localization of  $\text{Na}^+/\text{K}^+$ -activated ATPase in chloride cells from the gills of a euryhaline teleost. *Anat. Rec.*, 193: 99-130.
- Houston, A.H. (1985) Erythrocytic magnesium in freshwater fishes. *Magnesium*, 4: 106-128.
- Hunn, J.B. (1972) Blood chemistry values for some fishes of the upper Mississippi river. *J. Minn. Acad. Sci.*, 38: 19-21.



- Jensen, F.B. (1990) Nitrite and red cell function in carp: Control factors for nitrite entry, membrane potassium ion permeation, oxygen affinity and methaemoglobin formation. *J. Exp. Biol.*, 152: 149–166.
- Knox, D., C.B. Cowey, and J.W. Adron (1983) Studies on the nutrition of rainbow trout (*Salmo gairdneri*). Magnesium deficiency: The effect of feeding with a Mg-supplemented diet. *Br. J. Nutr.*, 50: 121–127.
- Mayer-Gostan, N., S.E. Wendelaar Bonga, and P.H.M. Balm (1987) Mechanisms of hormone actions on gill transport. In: *Vertebrate endocrinology: Fundamentals and biomedical implications*. Vol. 2. Regulation of water and electrolytes. P.K.T. Pang and M.P. Schreibman, eds. Academic Press, San Diego, pp. 211–238.
- Milligan, C.L., and C.M. Wood (1982) Disturbances in haematology, fluid volume distribution and circulatory function associated with low environmental pH in the rainbow trout, *Salmo gairdneri*. *J. Exp. Biol.*, 99: 397–415.
- Nanba, K., I. Kakuta, K. Uematsu, and S. Murachi (1987) Annual changes in the osmolarity and inorganic ion level ratios between urine and plasma in carp *Cyprinus carpio*. *Nippon Suisan Gakkaishi*, 53: 913–918.
- Ogino, C., and J.Y. Chiou (1976) Mineral requirements in fish. II. Magnesium requirement of carp. *Bull. Jap. Soc. Scient. Fish.*, 42: 71–75.
- Ogino, C., F. Takashima, and J.Y. Chiou (1978) Requirements of rainbow trout for dietary magnesium. *Bull. Jap. Soc. Scient. Fish.* (in Japanese, with English abstract), 44: 1105–1108.
- Shim, K.F., and S.H. Ng (1988) Magnesium requirement of the Guppy (*Poecilia reticulata* Peters). *Aquaculture*, 73: 131–141.
- Thillart, G. van den, A. van Waarde, H.J. Muller, C. Erkelens, A. Addink, and J. Lugtenburg (1989) Fish muscle energy metabolism measured by in vivo  $^{31}\text{P}$ -NMR during anoxia and recovery. *Am. J. Physiol.*, 256: R922–R929.
- Velden, J.A. van der, Z. Kolar, G. Flik, J.J.M. de Goeij, and S.E. Wendelaar Bonga (1989) Magnesium distribution in freshwater tilapia. *Mag. Bull.*, 11: 28–33.
- Velden, J.A. van der, J.A. Groot, G. Flik, P. Polak, and Z.I. Kolar (1990) Magnesium transport in fish intestine. *J. Exp. Biol.*, 152: 587–592.
- Velden, J.A. van der, Z.I. Kolar, G. Flik, P. Polak, J.J.M. de Goeij, and S.E. Wendelaar Bonga (1991a) Magnesium transport between carp and water studied with  $^{28}\text{Mg}^{2+}$  as radiotracer. *Appl. Radiat. Isot.*, 42: 347–352.
- Velden, J.A. van der, F.A.T. Spanings, G. Flik, C. Zegers, Z.I. Kolar, and S.E. Wendelaar Bonga (1991b) Growth rate and tissue magnesium concentration in adult freshwater tilapia, *Oreochromis mossambicus* (Peters), fed diets differing in magnesium content. *J. Fish Biol.*, 39: 83–91.
- Velden, J.A. van der, G. Flik, and S.E. Wendelaar Bonga (1992) Prolactin cell activity and ion regulation in tilapia, *Oreochromis mossambicus* (Peters): Effects of a low magnesium diet. *J. Fish Biol.* 40: 875–885.
- Wendelaar Bonga, S.E., C.J.M. Lowik, and J.C.A. van der Meij (1983) Effects of external  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  on branchial osmotic water permeability and prolactin secretion in the teleost fish *Sarotherodon mossambicus*. *Gen. Comp. Endocrinol.*, 52: 222–231.
- Wendelaar Bonga, S.E., P.H.M. Balm, and G. Flik (1988) Control of prolactin secretion in the teleost *Oreochromis mossambicus*: Effect of water acidification. *Gen. Comp. Endocrinol.*, 72: 1–12.
- Wendelaar Bonga, S.E., G. Flik, P.H.M. Balm, and J.C.A. van der Meij (1990) The ultrastructure of chloride cells in the gills of the teleost *Oreochromis mossambicus* during exposure to acidified water. *Cell Tissue Res.*, 259: 575–585.